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Relationship of Plant Growth and Chlorophyll Content in Field-grown Rice

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Abstract

Changes of chlorophylls and fresh weight in leaf blade and whole plant of 5 rice genotypes were measured at the experimental farm of Taiwan Agricultural Research Institute during the first (1^{st}) and the second (2^{nd}) cropping seasons of 1998. The objectives were to study the correlation between chlorophyll content and plant growth and its seasonal variation. The ratio of chlorophyll a to chlorophyll b was also determined to clarify the role of pigmentation relationship during rice growth. It was shown that rice plants began with lower chlorophyll content, increased as plant develops, and decreased when plant aged. Pattern for seasonal chlorophyll variation is best fitted by the Gaussian function, with the determining factors (R^2) greater than 0.89. The total chlorophyll was higher in the 2^{nd} crop than in the 1^{st} crop and level of chlorophyll content was higher in the whole plant than in leaf blade. After excluding the influence of growth rate between crops, Gaussian function is also fitted to seasonal changes in total chlorophyll on the unit time baseline. Seasonal changes of fresh weight in leaf blade and whole plant followed a similar best fit curve pattern to the total chlorophyll. Plants grown in the 2^{nd} crop had higher fresh weight values than those grown in the 1^{st} crop at the relative transplanting days and unit time baseline. By regression analyses, it revealed a closed positive correlation (R^2>0.82) between fresh weight and total chlorophyll. The linear relationship shows that the amount of total chlorophyll had a pronounced effect on values of fresh weight in both cropping seasons. However, at the same chlorophyll content, rice plants grown in the 1^{st} crop supported a higher fresh weight than those grown in the 2^{nd} crop. By further analyze leaf and whole plant pigmentation (chlorophyll a and chlorophyll b) during plant growth, it was found that the ratio of chlorophyll a/b was a linear function, decreasing after transplanting. Plants grown in the 1^{st} crop had higher values in green-to- yellow pigment ratio than those grown in the 2^{nd} crop.

Key words: rice (*Oryza sativa* L.), fresh weight, plant growth, total chlorophyll, pigmentation relationship.

田間水稻葉綠素含量與生長之關係

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摘要：本文研究旨在追蹤田間水稻葉片及全株葉綠素含量與鮮重之季節變化，據以評估葉綠素與植株生長之關係，並由葉率素a 與葉綠素 b 比例之季節變化澄清色素比例所扮

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演的角色。田間試驗係於行政院農業委員會農業試驗所農場進行，包括1998年一期作及二期稻作。試驗結果顯示，無論葉片或全株，葉綠素總量自生長初期開始增加至抽穗前達頂點再隨著成熟老化遞減。葉綠素總量在一、二期作的季節變化適用於Gaussian函數，決定係數(R^2) 超過0.89 。葉綠素總量在二期作全期皆高於一期作者，且全株葉綠素總量大於葉片葉綠素總量。經利用單位時間標準化轉換消除生長速率差異後，葉綠素總量季節變化仍適用於Gaussian函數，且在單位時間刻度上二期作稻株葉綠素總量依然高於一期作者。稻株葉片與全株鮮重在一、二期作期間亦呈現類似於葉綠素總量之Gaussian函數曲線，且同時期二期作稻株鮮重也大於一期作稻株鮮重。經過回歸分析後發現，無論葉片或全株，鮮重與葉綠素總量之間呈現直線相關 (R^2>0.82)，顯見葉綠素總量在兩期作均顯著影響鮮重的表現。惟在相同的葉綠素總量下，一期作稻株具有較高的植體鮮重。又葉綠素a 及葉綠素b 比例在一、二期作生育期間呈現直線遞減趨勢，然一期作稻株具有較高的葉綠素 a/b 比值。

關鍵詞：水稻(*Oryza sativa* L.)、鮮重、植株生長、葉綠素含量、色素比例。

Introduction

Chlorophylls, especially chlorophyll a and chlorophyll b in higher plants, are the primary pigments intercepting solar energy and acting as power plant in the processes of photosynthesis. It is well known that chlorophyll content and photosynthetic rate is positively correlated (Buttery and Buzzell, 1977; Buttery *et al.*, 1981; Earl and Tollenaar, 1997) and the relationship is affected by light intensity (Hesketh, 1963). High chlorophyll content increases absorption of radiation and stimulates higher photosynthetic rate at high irradiance than at low irradiance (Sestak, 1966). With higher level of chlorophyll content during the growing period, therefore, it can be expected to have a more vigorous growth.

The concentration of chlorophyll reflects the status of nitrogen in a plant and is therefore widely used in assessing the requirement of nitrogen fertilizer in many crops (Bullock and Anderson, 1998; Johnkutty and Palaniappan, 1996; Ma *et al.*, 1996; Ladha *et al.*, 1998; Peng *et al.*, 1995; Turner and Jund, 1991; Wood *et al.*, 1992). As nitrogen content and photosynthetic rate are closely related (Yoshida and Coronel, 1976; Uchida *et al.*, 1982), maintaining adequate leaf chlorophyll throughout the growing period may be necessary for a better growth and yield (Turner and Jund, 1991; Wood *et al.*, 1992; Yoshida, 1981). In rice, leaf chlorophyll content was found to highly correlated with leaf and shoot nitrogen (Johnkutty and Palaniappan, 1996; Ladha *et al.*, 1998; Peng *et al.*, 1995) and yield (Johnkutty and Palaniappan, 1996). However, no study has been conducted to direct link the correlation between growth and chlorophyll content in field-grown rice, particularly in a two crops system such as Taiwan.

In this study, changes in chlorophyll content during the growing period of rice was examined, and the relationship between chlorophyll content and fresh weights of leaf and whole plant were analyzed. The importance of chlorophyll in relation to plant growth was also discussed between different cropping seasons.

Materials and Methods

Seedlings of 5 rice (*Oryza sativa* L.) genotypes were transplanted north-south bound

in the experimental farm of Taiwan Agricultural Research Institute at Wufeng, Taiwan on a loam soil (Table 1). The soil had a pH of 5.36 and organic matter of 2.21%. The experimental plot was laid out in a complete randomized design (CRD) with 4 replications. The subplot size was 144 m^2 (30 m × 4.8 m), and plants (hills) were spaced 0.3 m × 0.18 m apart with 2-3 plants per hill.

Rice plants received ammonium sulphate (21% granule, Taiwan Fertilizer Company (TFC), Kaohsiung, Taiwan) at a rate of 200 kg ha^{-1} at 10 days after transplanting (DAT). A composite fertilizer Taifei-39 (granule, $N:P_2O_5:K_2O$=12%:18%:12%, TFC; 200 kg ha^{-1}) was added at 28 DAT, and urea (46% granule, TFC; 100 kg ha^{-1}) was sprayed at 1-2 weeks before heading. Herbicide butaclor-benthiocarb 8% granule (5% • S-(4-Chlorobenzyl)-N • N-diethylthiocarbamate; 3%, 2-chloro-2,6-diethyl-N-(butoxymethyl)acetanilide) was applied at a rate of 30 kg ha^{-1} in the first week after transplanting. Weed control was practiced as hand-weeding at 28 DAT, right before the second fertilization application. Triphenyltin acetate (2% granule, 15-20 kg ha^{-1}) was applied as needed for pest control.

Total chlorophyll (Chlorophyll a plus chlorophyll b) and pigment ratio of chlorophyll a to chlorophyll b (chlorophyll a/b) in leaf blade and whole plant (hill) were analyzed periodically after transplanting to monitor changes during the growing seasons. Chlorophyll was analyzed by the method of Arnon (1949). Generally 4 hills from each replication were sampled in each sampling for chemical analysis and mean value was used for statistics. After a small amount of leaf discs were used to determine chlorophyll content, the rest leaves and shoots were mixed as a composite sample for chlorophyll estimation of whole plant. Sample materials were ground with sea sand and liquid nitrogen. The mixed solution was extracted with 80% acetone and centrifuged at 2500 rpm for 10 min. The upper portion of supernatant was used for estimation of chlorophylls by the following formulae:

$$\text{chlorophyll a (mg)}=\frac{(12.7 \times D_{663}-2.69 \times D_{645}) \times V}{1000 \times W} \quad .....(1)$$

$$\text{chlorophyll b (mg)}=\frac{(22.9 \times D_{645}-4.68 \times D_{663}) \times V}{1000 \times W} \quad .....(2)$$

$$\text{Total chlorophyll (mg)}=\frac{D_{652} \times V}{34.5 \times W} \quad ....(3)$$

where $D\lambda$ is the absorbance readings at wavelength λ (nm), V is the total volume of supernatant by 80% acetone extraction (ml), and W is the fresh weight of leaf blade or whole plant (g). In each sampling, another 6 hills were harvested from each subplot for determination of fresh weights in leaf blade and whole plant.

The unit time transformation is determined by setting the life span to 100 scale and then

Table 1. Some characters of rice genotypes used in this experiment.

Abbreviation	Rice genotype	Heading date		Life span@	
		1st crop	2nd crop	1st crop	2nd crop
TK8	Tai-Ken 8	5/14	9/30	128	108
TCS10	Taichung Sen 10	5/14	10/07	128	108
TN1	Taichung Native 1	5/08	10/07	128	108
TNG67	Tainung 67	5/14	9/30	128	108
BN	TARI-G-001	5/08	10/07	128	108

@ days from transplanting to harvest.

Transplanting date: 02/12/98, the 1st crop of 1998; 08/04/98, the 2nd crop of 1998.

dividing to growing days of the respective cropping season (Table 1). The technique is applied to compare differences in chlorophyll content between two cropping seasons under the same unit time baseline. The experimental data were analyzed with proper statistics.

Results and Discussion

Measurements of total chlorophyll (chlorophyll a plus chlorophyll b) in leaf blade and whole plant (the aboveground plant parts) from 5 rice genotypes grown in the 1st and the 2nd cropping seasons of 1998 were pooled and graphed in Figure 1. It was shown that rice plants began with lower chlorophyll content, increased as plant develops, and decreased when plant aged. As nitrogen fluctuation produced the changes in chlorophyll content (MacNeil *et al.*, 1987), changes of chlorophyll may imply a seasonal variation in nitrogen during the growth of rice. Pattern for seasonal chlorophyll variation is best fitted by the Gaussian function, with the determining factors (R^2) greater than 0.89. The values of R^2 for the individual genotypes in two crops were listed in Table 2, all greater than 0.91. The total chlorophyll reached maximum values at about 73 and 88 days after transplanting for the 2nd and the 1st crops, respectively, in both leaf blade and whole plant. Figure 1 further indicated that, rice plants induced promptly the chlorophyll content from the early stage of the 2nd crop relative to that of the 1st crop, and maintained such difference throughout the growing season. Thus, total chlorophyll was higher in the 2nd crop than in the 1st crop. Moreover, level of total chlorophyll was higher in the whole plant than in leaf blade.

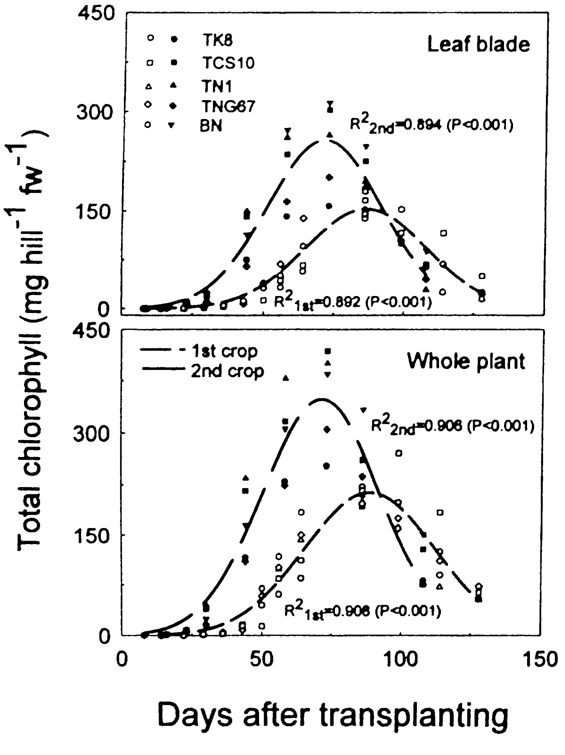

Fig. 1. Changes of total chlorophyll in leaf blade and whole plant of 5 rice genotypes during the 1st and 2nd cropping seasons of 1998. The open symbols are genotypes measured in the 1st crop and the closed symbols are those measured in the 2nd crop.

As the growing periods were different between the 1st and the 2nd crops, the unit time transformation technique was applied so that the difference in chlorophyll content between cropping seasons may be compared on the unit time baseline. After excluding the influence of growth rate between crops, results show that Gaussian function is also fitted to seasonal changes in total chlorophyll (Fig. 2 and Table 3). The total chlorophyll was higher in the 2nd crop than in the 1st crop at the same unit time scale, and the maximal chlorophyll content was located near heading in both crops. It is therefore can be concluded that, on a per hill basis, plants grown in the 2nd cropping season had greater level of chlorophyll than those grown in the 1st cropping season. The results comply with the higher growth rate observed in the 2nd cropping season. This finding was true in all of the tested genotypes despite a wide range of parameter values (Table 3).

Seasonal changes of fresh weights in leaf blade and whole plant followed a similar best fit curve pattern to the total chlorophyll (Fig. 3) for

Table 2. Equations of Gaussian function fitted for chlorophyll concentration (mg hill^{-1} fw^{-1}) measured after transplanting in different rice genotypes grown in the 1st and the 2nd cropping seasons of 1998.

Rice genotype	Gaussian function $Y=a*\exp\{-0.5*[(x-x_0)/b]\}$			R^2
	a	b	x_0	
1st crop				
Leaf blade				
TK8	151.00	19.81	90.77	0.984 (P<0.001)
TCS10	150.67	22.96	93.32	0.949 (P<0.001)
TN1	181.20	16.38	81.70	0.991 (P<0.001)
TNG67	158.11	20.91	82.19	0.939 (P<0.001)
BN	155.90	24.14	85.46	0.911 (P<0.001)
Whole plant				
TK8	219.13	20.27	89.46	0.984 (P<0.001)
TCS10	247.89	22.78	95.13	0.959 (P<0.001)
TN1	204.99	21.76	84.21	0.971 (P<0.001)
TNG67	204.20	24.44	87.74	0.956 (P<0.001)
BN	221.41	23.92	85.11	0.932 (P<0.001)
2nd crop				
Leaf blade				
TK8	181.15	22.84	76.47	0.971 (P<0.001)
TCS10	299.47	20.40	71.28	0.991 (P<0.001)
TN1	290.72	19.08	67.54	0.989 (P<0.001)
TNG67	203.35	19.17	72.04	0.994 (P<0.001)
BN	322.59	20.17	72.37	0.987 (P<0.001)
Whole plant				
TK8	266.83	21.64	72.71	0.985 (P<0.001)
TCS10	392.96	21.43	70.49	0.962 (P<0.001)
TN1	422.93	18.34	65.59	0.981 (P<0.001)
TNG67	300.09	20.03	73.27	0.996 (P<0.001)
BN	389.83	22.58	75.07	0.989 (P<0.001)

rice genotypes grown in 1998. The parameters of Gaussian function for different rice genotypes in two cropping seasons were listed in Table 4. Again, plants grown in the 2nd crop had higher fresh weight values than those grown in the 1st crop at the relative transplanting days and unit time baseline (data not shown). By regression analyses, it revealed a closed positive correlation (R^2>0.82) between fresh weight and total chlorophyll (Fig. 4). A simple linear model can best describe the relationship between fresh weight and total chlorophyll in both leaf blade and whole plant. The relationship shows that the amount of total chlorophyll had a pronounced effect on values of fresh weight in both cropping seasons, i.e., seasonal changes of chlorophylls were in compatible with the growth patterns of vegetative tissues. The fact also suggests that growth of rice plants acquires adequate chlorophyll to provide sufficient photosynthetic assimilates needed for growth, and chlorophyll measurements can therefore be useful in estimating plant growth of a rice crop during the growing season.

However, at the same chlorophyll content, rice plants grown in the 1st crop supported a higher fresh weight than those grown in the 2nd crop. It implies that rice plant grown in the 1st

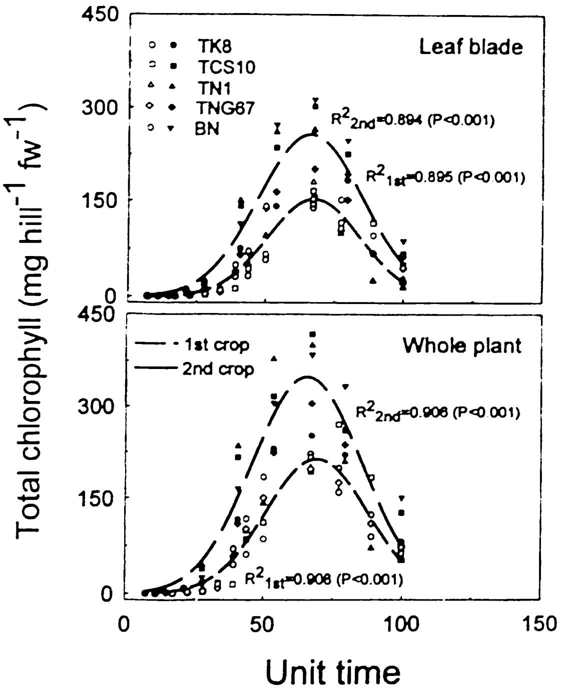

Fig. 2. Changes of total chlorophyll in leaf blade and whole plant of 5 rice genotypes on the unit time baseline during the 1st and 2nd cropping seasons of 1998. The open symbols are genotypes measured in the 1st crop and the closed symbols are those measured in the 2nd crop.

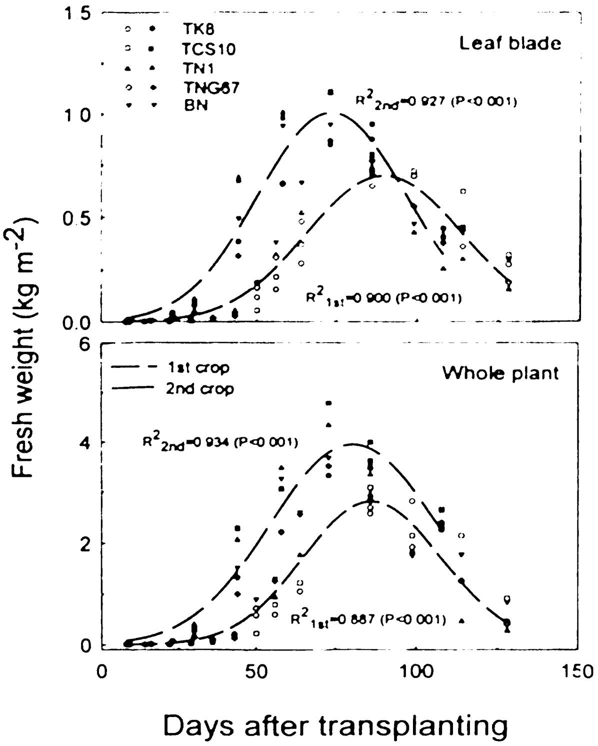

Fig. 3. Changes of fresh weight in leaf blade and whole plant of 5 rice genotypes during the 1st and 2nd cropping seasons of 1998. The open symbols are genotypes measured in the 1st crop and the closed symbols are those measured in the 2nd crop.

crop had a more vigorous photosynthetic efficiency and hence an elevated biomass formation during the course of growing season. In soybean, photosynthetic rate was found highly correlated with chlorophyll content on a unit area basis (Buttery and Buzzel, 1977). Good agreement was also shown between mean net assimilation rate and specific leaf weight on a whole-plant basis (Buttery *et al.*, 1981). Based on the present results, differentiation of rice growth between cropping seasons from fresh weight-total chlorophyll relationship is possible, and adjustment of chlorophyll content by appropriate cultural practices to improve biomass production is feasible. The use of this technique provides an alternative to estimate plant growth and nutritional management.

By further analyze leaf and whole plant pigmentation (chlorophyll a and chlorophyll b) during plant growth, it was found that the ratio of chlorophyll a to chlorophyll b (chlorophyll a/b) was a linear function (Fig. 5). Since chlorophyll a and chlorophyll b changed in parallel (data not shown) and the ratio of chlorophyll a/b was decreasing after transplanting, it indicated a substantial decrease in the green-to-yellow pigment relationship toward maturity. Others have pointed out that one of the characteristics of plants acclimated to low irradiance is a reduction in the ratio of chlorophyll a/ b with an increase in the relative amount of chlorophyll b (Anderson, 1986; Bjorkman, 1981; Evans, 1988). The decline in chlorophyll content is also retarded at low irradiance (Hidema *et al.*, 1991,1992) and under chill and heat stresses (Tewari and Tripathy,

Table 3 Equations of Gaussian function fitted for chlorophyll concentration (mg $hill^{-1}$ fw^{-1}) measured after transplanting on unit time baseline in different rice genotypes grown in the 1st and the 2nd cropping seasons of 1998

Rice genotype	Gaussian function $Y=a*exp\{-0.5*[(x-x_0)/b]\}$			R^2
	a	b	x_0	
1st crop				
Leaf blade				
TK8	151.00	15.48	70.91	0.984 ($P<0.001$)
TCS10	150.67	17.94	72.91	0.949 ($P<0.001$)
TN1	181.20	12.80	63.83	0.991 ($P<0.001$)
TNG67	158.11	16.34	64.21	0.939 ($P<0.001$)
BN	155.90	18.86	66.77	0.911 ($P<0.001$)
Whole plant				
TK8	219.13	15.84	69.89	0.984 ($P<0.001$)
TCS10	247.89	17.80	74.32	0.959 ($P<0.001$)
TN1	204.99	17.00	74.32	0.971 ($P<0.001$)
TNG67	204.20	19.10	68.55	0.956 ($P<0.001$)
BN	221.41	18.69	66.49	0.932 ($P<0.001$)
2nd crop				
Leaf blade				
TK8	181.15	21.14	70.80	0.971 ($P<0.001$)
TCS10	299.47	18.89	66.00	0.991 ($P<0.001$)
TN1	290.72	17.66	62.54	0.989 ($P<0.001$)
TNG67	203.35	17.75	66.70	0.994 ($P<0.001$)
BN	322.59	18.68	67.01	0.987 ($P<0.001$)
Whole plant				
TK8	266.83	20.03	67.32	0.985 ($P<0.001$)
TCS10	392.96	19.85	65.27	0.962 ($P<0.001$)
TN1	422.93	16.98	60.73	0.981 ($P<0.001$)
TNG67	300.09	18.55	67.84	0.996 ($P<0.001$)
BN	389.83	20.91	69.51	0.989 ($P<0.001$)

1998).

Plants grown in the 1st crop had higher values in green-to-yellow pigment ratio than those grown in the 2nd crop (Fig. 5). Such consistent difference in the green-to-yellow pigment relationship throughout the growing season therefore made it suitable to explain seasonal changes in plant growth between two crops as previously mentioned. The results also suggest that a field-portable chlorophyll meter would have practical applications in estimating and monitoring rice growth or evaluating the advantages of cultural practices. However, calibration of field chlorophyll measurements will be required prior to routine application for chlorophyll determination and nutrient management.

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Table 4. Equations of Gaussian function fitted for fresh weight (kg m^{-2}) measured after transplanting in different rice genotypes grown in the 1st and the 2nd cropping seasons of 1998.

Rice genotype	Gaussian function $Y=a*exp\{-0.5*[(x-x_0)/b]\}$			R^2
	a	b	x_0	
1st crop				
Leaf blade				
TK8	0.73	22.10	93.71	0.991 (P<0.001)
TCS10	0.77	24.32	96.32	0.987 (P<0.001)
TN1	0.69	21.33	83.06	0.953 (P<0.001)
TNG67	0.66	23.54	87.26	0.969 (P<0.001)
BN	0.75	24.55	85.94	0.881 (P<0.001)
Whole plant				
TK8	2.80	19.81	90.77	0.984 (P<0.001)
TCS10	2.79	22.96	93.32	0.959 (P<0.001)
TN1	3.36	16.38	81.70	0.991 (P<0.001)
TNG67	2.93	20.91	82.19	0.939 (P<0.001)
BN	2.82	24.09	84.88	0.888 (P<0.001)
2nd crop				
Leaf blade				
TK8	0.91	24.27	78.66	0.988 (P<0.001)
TCS10	1.16	23.00	72.22	0.971 (P<0.001)
TN1	1.15	20.34	67.74	0.975 (P<0.001)
TNG67	0.88	22.72	76.77	0.991 (P<0.001)
BN	1.01	23.45	73.23	0.958 (P<0.001)
Whole plant				
TK8	3.64	25.12	83.10	0.992 (P<0.001)
TCS10	4.58	25.47	79.09	0.966 (P<0.001)
TN1	4.06	25.19	75.51	0.930 (P<0.001)
TNG67	3.69	24.66	83.80	0.992 (P<0.001)
BN	3.94	26.87	81.26	0.962 (P<0.001)

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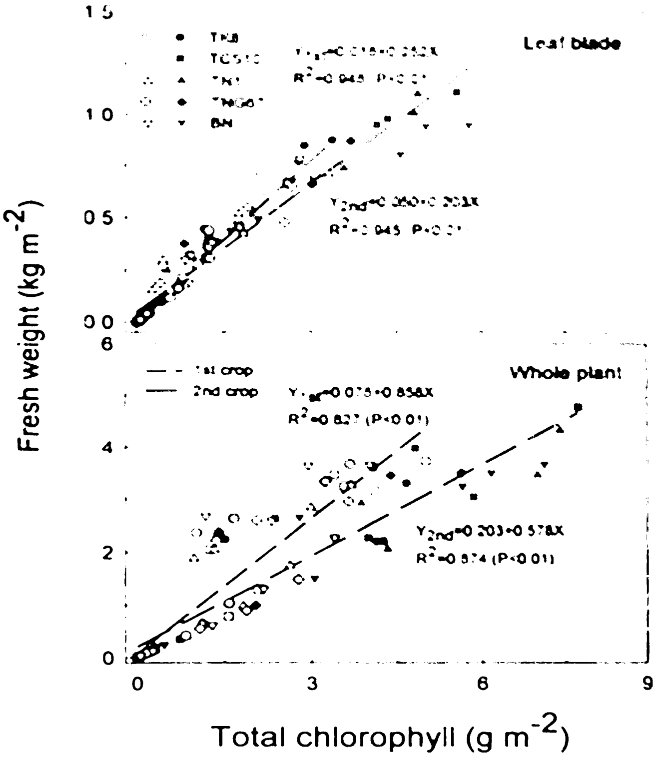

Fig. 4. Linear correlation between fresh weight and total chlorophyll in leaf blade and whole plant of 5 rice genotypes grown in the 1st and 2nd cropping seasons of 1998. The open symbols are genotypes measured in the 1st crop and the closed symbols are those measured in the 2nd crop.

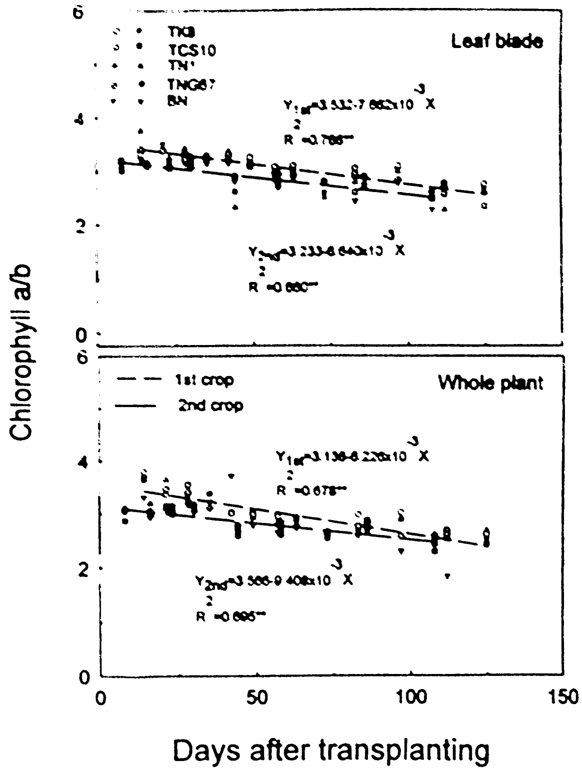

Fig. 5. Changes of the ratio of chlorophyll a to chlorophyll b (chlorophyll a/b) in leaf blade and whole plant of 5 rice genotypes during the 1st and 2nd cropping seasons of 1998. The open symbols are genotypes measured in the 1st crop and the closed symbols are those measured in the 2nd crop.

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